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14. ABSTRACT

Forty three paraffin fixed tissue samples from anonymous hereditary and non-hereditary breast cancer cases were selected for the study. Five µm histological sections were prepared on microscopic slides for processing and analyses. All of the 43 tissue samples (11 nonhereditary; 17 with a BRCA1 mutation; 15 with a BRCA2 mutation) were hybridized and imaged for telomere and centromere. Data recording on telomere organization and distribution are in progress. This work is expected to be completed by the end of 2005. Since we are using centromere signal intensity as a standard for relative fluorescence, we expect to complete the similar 3D image data obtained from the centromere signals obtained from these patient samples around the same time. We have data on telomere sizes and their frequency distribution patterns in the tumor cells of hereditary and non-hereditary breast cancer patients. We are currently doing statistical tests on these data to verify the sensitivity and effectiveness of our 3D measurement system. Our early results from studies using 3D telomere imaging system and quantization on cell lines with a *BRCA1* mutation (HCC1937) and with a *BRCA2* mutation (Capan-1) shows presence of significantly larger telomere aggregates in comparison to the telomeres of wild type (MCF-7) cells ($p < .0001$ for comparisons between *BRCA1* or *BRCA2* and MCF-7). One hundred nuclei from each of MCF7, HCC1937 and Capan-1 have been examined and according to our data there are statistically significant differences in telomere sizes and their distribution patterns between the *BRCA* mutated HCC1937, Capan1 and wild type MCF7 cells. Our planned work on c-Myc deregulation has not commenced but we expect to start this in the near future.

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Breast cancer in three dimensions: Genomic instability and Hereditary Breast Cancer

Dr William Foulkes, Dr Sabine Mai

Investigator Award: W81XWH-04-1-0783

Introduction

In this study, we were funded to find out whether disorganization of telomeres in *BRCA1* and/or *BRCA2*-related breast cancer is a key step in the development of these cancers. We took advantage of very new technology to make significant inroads into our understanding of the organization of these chromosomal structures and their role, if any, in hereditary breast cancers.

We set out to achieve three main objectives: **1)** To define the frequencies and 3-D volumes of telomeric aggregations in *BRCA1*, *BRCA2* and non-hereditary breast cancers. We plan to study at least ten tumors will be studied per group. Early-stage invasive cancers and ductal carcinoma *in situ* will be included. **2)** To investigate if regulatable *BRCA1/2* mutations in cell lines alter the levels of telomeric aggregation and genomic instability in 3-D nuclei. **3)** To investigate whether *BRCA1* or *BRCA2* mutations present in cell lines, together with c-Myc deregulation, accelerate telomeric aggregation/genomic instability in 3-D nuclei.

Body of Text

Task 1: To define the frequencies and 3-D volumes of telomeric aggregations in BRCA1, BRCA2 and non-hereditary breast cancers. Ten tumors will be studied per group.

- Forty-three paraffin fixed tissue samples from anonymous hereditary and non-hereditary breast cancer cases were selected for the study. Five μm histological sections were prepared on microscopic slides for processing and analyses.

- All of the 43 tissue samples (11 nonhereditary; 17 with a *BRCA1* mutation; 15 with a *BRCA2* mutation) were hybridized and imaged for telomere and centromere. Data recording on telomere organization and distribution are in progress. We present a short account of this data in Figures 1a & 1b (see appendix). This work is expected to be completed by the end of 2005. Since we are using centromere signal intensity as a standard for relative fluorescence, we expect to complete the similar 3D image data obtained form the centromere signals from these patient samples around the same time.

- Both telomere and centromere hybridizations were specific as shown by metaphase hybridizations and the correct number of the telomeric signals observed at the ends of chromosomes prepared from primary cells (Figure 2 in appendix).

- Work so far completed on 23 out of 43 patient samples: we have data on telomere sizes and their frequency distribution patterns in the tumor cells of hereditary and non-hereditary breast cancer patients. We are currently doing statistical tests on these data to verify the sensitivity and effectiveness of our 3D measurement system. Full scale analyses of all patient data will be presented at the end of this study. Figures 1a & 1b (see appendix) shows an overview of the telomere frequency distribution pattern of telomere signal intensity from three representative hereditary and non-hereditary breast cancer patients showing characteristics differences in *BRCA1* mutated and non-*BRCA* mutated cases. Once all of the patient samples are analyzed a Kruskal-Wallis Test will be performed to test the null hypothesis that there is no difference in the distribution of the intensities of the telomeric signals between each group. At that point, we will be able to present the entire set of data in a composite format.

Task 2: To investigate if regulatable BRCA1/2 mutations in cell lines alter the levels of telomeric aggregation and genomic instability in 3-D nuclei.

- **Preliminary Results:** Our early results from studies using 3D telomere imaging system and quantization on cell lines with a *BRCA1* mutation (HCC1937) and with a *BRCA2* mutation (Capan-1) shows presence of significantly larger telomere aggregates in comparison to the telomeres of wild type (MCF-7) cells ($p < .0001$ for comparisons between *BRCA1* or *BRCA2* and MCF-7). The respective sizes (average ±SE) of telomeres in voxel values were 356398.4 ±46891.8 (HCC1937), 274570.6 ±21670.6 (Capan-1) and 99064.5 ±11872 (MCF-7)(table 1 in appendix). Our ongoing work on biopsy samples from hereditary and non-hereditary breast cancer cases shows similar trends.

- 100 nuclei form each of MCF7, HCC1937 and Capan1 are examined and according to our data presented in table 1 and Figures 3a and 3b. There are statistically significant differences in telomere sizes and their distribution patterns between the BRCA mutated HCC1937, Capan1 and wild type MCF7 cells.

- 28N (*BRCA2* mutation) and HNME cell lines have been hybridized using telomere and centromere probes and the data obtained from these two normal cell lines analyzed. We are currently working on the 28T (*BRCA2* mutation) cell line and only after completing it, we can make a comparison.

- Studies involving regulation of *BRCA1/2* levels have not yet commenced.

Task3: To investigate whether BRCA1 or BRCA2 mutations present in cell lines, together with c-Myc deregulation, accelerate telomeric aggregation/genomic instability in 3-D nuclei.

- We have been unable to commence this work as were are still working on the cell line telomeric aggregation but we should be commencing this work shortly.

Key Research Accomplishments

- Forty-three tissue samples (11 nonhereditary; 17 with BRCA1 mutation; 15 with BRCA2 mutation) were hybridized and imaged for telomere and centromere. Data analysis is complete for twenty-three of the forty-three samples.

- HCC1937 (*BRCA1* mutation) shows presence of significantly larger telomere aggregates in comparison to the telomeres of wild type (MCF-7) cells (Figures 3a & 3b in appendix). The respective sizes of telomeres in voxel values were 356398.4 ±46891.8 (HCC1937), 274570.6 ±21670.6 (Capan-1) and 99064.5 ±11872 (MCF-7). These differences are highly significant. Our ongoing work on biopsy samples from hereditary and non-hereditary breast cancer cases shows similar trends.

Reportable Outcomes

- Canadian Telomere Group. ***Three Dimensional Organization of Telomeres in Human Breast Cancer.*** Poster Presentation. Soumya Panigrahi, Landon Wark, Bart Vermolen Peter Watson, Linda Snell, Alice Ya-Chun Chuang, Kimberley Kotar, Yuval Garini, WilliamD.Foulkes, Sabine Mai. Lennoxville, Qc. June 2004

- U.S Army Era of Hope Conference. ***Breast cancer in three dimensions: genomic instability and hereditary breast cancer.*** Poster Presentation. Kimberley Kotar, Soumya Panigrahi, Louis R Bégin, Yuval Garini, Sabine Mai, William D. Foulkes. Philedelphia, PA. June 2005.

- PI Foulkes and Co-I Mai Received funding for ***BRCA1, CDC4, Cyclin E, and chromosomal instability in breast cancer*** from Canadian Breast Cancer Research Alliance from results generated by this study, June 2005

Conclusions

Based on our recent work, it has become possible to examine the presence or absence of telomeric aggregates in hereditary breast cancers (suggesting a genomic instability in individual interphase nuclei and tissue) without the need to examine metaphases. Larger scale studies on paraffin-embedded samples of breast cancers may open new avenues towards rapid and precise characterization of the chromosomal organization of breast cancer at a three-dimensional level. This new modality of monitoring genomic instability could potentially have impact on the future studies in cancer biology, genetics and diagnostics.

References

- **Nil**

Appendices

Figure 1: 3D Telomere signal intensity distribution pattern in patients with hereditary [BRCA1 (P1) BRCA2 (P6)] and non-hereditary breast cancer (P43)*.

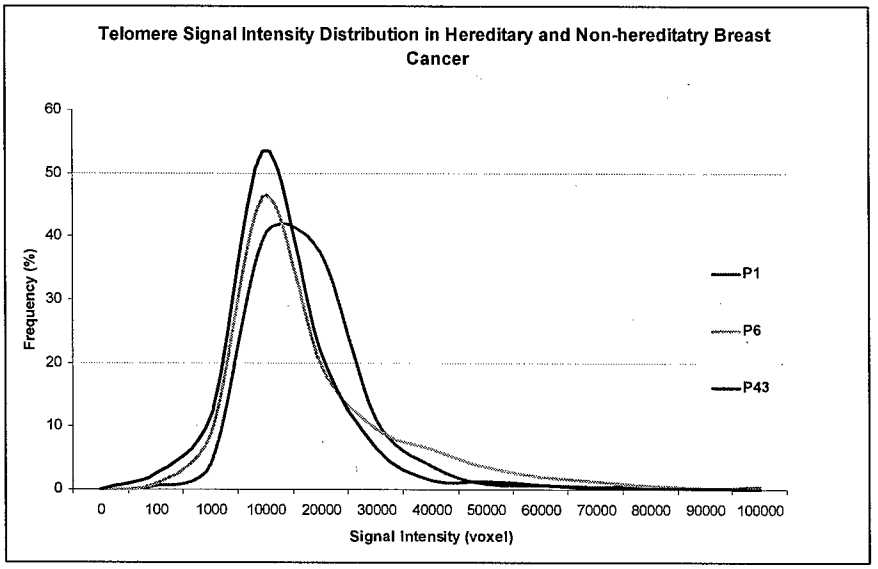
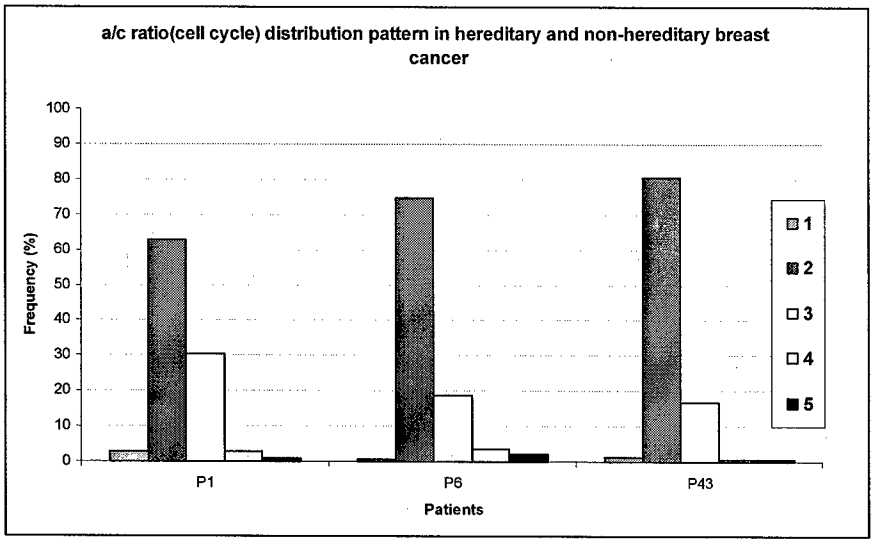


Fig 1a. Frequency distribution (FD) pattern of telomere signal sizes in voxel showing a right shift of the distribution curve in BRCA1 mutated P1 in comparison to the FD pattern of P6 and P43.



1b. Cell cycle distribution pattern in the analyzed nuclei of hereditary and non-hereditary breast cancers: A higher a/c ratio implies that the cell is closer to G2 phase.

** Data from three representative patient samples out of 43 case samples under current investigation are presented here.*

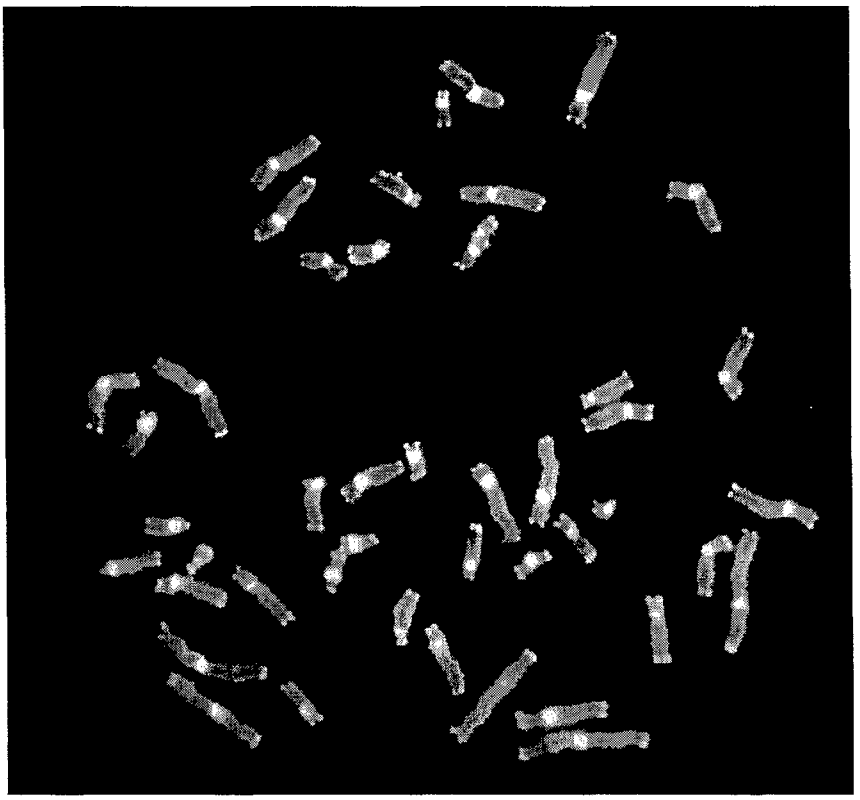
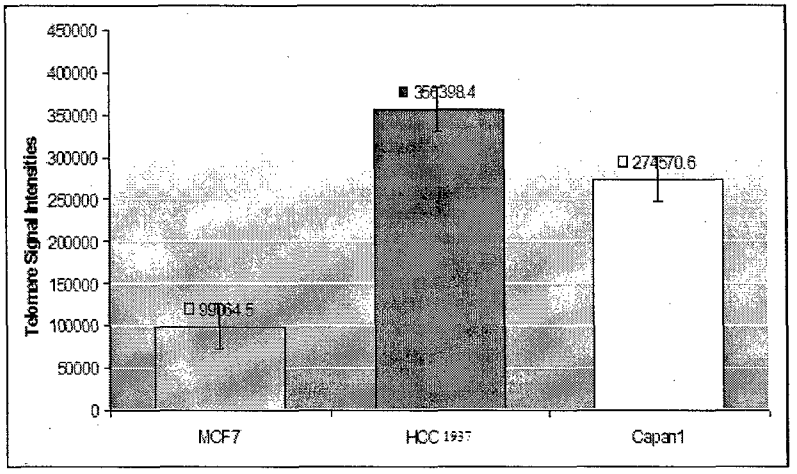
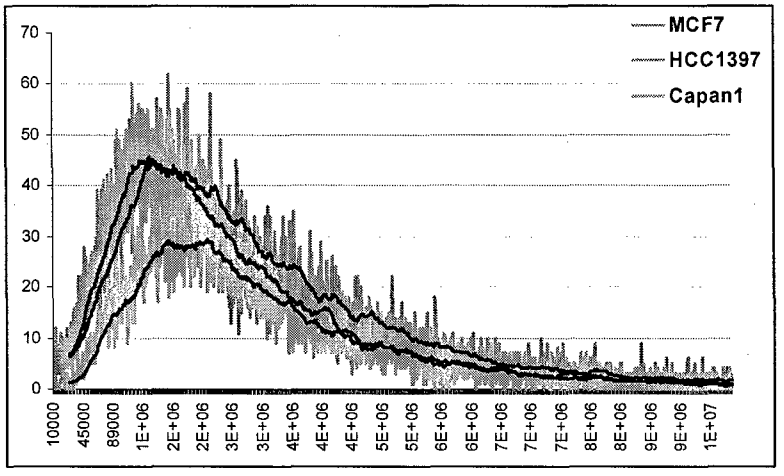


Figure 2 Normal human lymphocyte in metaphase: Metaphase spread was hybridized with PNA probe, conjugated with Cy3 and FITC for telomeres and the centromeres respectively. Rest of the DNA in each chromosome is stained with DAPI (blue).

Figure 3 Telomere signal intensity values in three human breast cancer cell lines. MCF7 (WT), HCC1937 (BRCA1 mutant) and Capan1 (BRCA2 mutant) with significant differences.



3a. Average telomere signal intensity in voxel values



3b. Frequency distribution pattern of telomere signal intensity.

Table 1
Average telomere signal intensity values for three human breast cancer cell lines showing significant differences in between the BRCA mutant and non-mutants.

	Total # of Nuclei Studied	Average # of detected Telomeres/nucleus	Average Intensity of the Telomeres	Standard Errors
MCF7 (WT)	100	71	99064.5	11872
HCC1397 (BRCA1)	95	78	356398.4	46891.81
Capan1 (BRCA2)	100	71	274570.6	21670.58